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A Study of Carbon Tetrachloride

IV. Esterase Distribution in Liver and Sera of Rats Exposed to CCl_4 Vapors[1]

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Recent reports (Cornish and Block, 1960a, b) have demonstrated the intimate relationship between certain serum and liver enzyme levels following the exposure of animals to carbon tetrachloride vapor. Serum esterase, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) all are markedly increased 24 hours after exposure to 6000 ppm of CCl_4 vapor for 4-6 hours. Serum GOT may reach values up to 55 times those of control animals following such an exposure. Fleisher and Waki (1961) have recently demonstrated by electrophoretic means the presence of a new GOT enzyme in the serum of dogs given oral doses of CCl_4. Serum esterase levels of rats exposed to 6000 ppm of CCl_4 may be nearly doubled, but these elevated levels rapidly return to normal and below normal values. Liver esterase values in exposed rats drop to extremely low levels. The exact relationship of serum enzyme levels to liver enzyme levels in control and exposed rats is not always clear. If one assumes a loss of enzyme from damaged tissue into the serum one would expect a reciprocal relationship to exist depending upon the time the samples were taken, the rate of removal or inactivation of the enzyme in the serum, and the rate of resynthesis of enzyme in damaged tissue. In addition it is difficult to assess the role of activation or inactivation of serum or liver enzymes by CCl_4 or its metabolic products. The present study makes use of paper electrophoresis in an attempt to clarify serum and tissue enzyme relationships.

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METHODS

Paper electrophoresis was carried out using the Spinco model R electrophoretic apparatus. All runs were for 16 hours at 4.2 ma using 2 cells and Veronal buffer, pH 8.6. Paper strips were stained for protein with Amidoschwartz (1952) and for esterase activity by the Gomori technique (1952) using α-naphthyl acetate as the substrate.

Male rats of the Sprague-Dawley strain (Holtzman) weighing 250-300 g were used in all studies. Animals were exposed to CCl_4 vapor in a dynamic exposure chamber as previously described (Cornish and Block, 1960a). The rats were sacrificed by stunning and decapitation. Blood was collected and the serum separated and kept frozen until utilized. The livers were removed, rinsed, blotted dry, and weighed; portions were immediately homogenized with one volume of distilled water. The homogenate was centrifuged at low speed to remove cellular debris, and the supernate was removed and frozen until used.

RESULTS

As has been previously reported (Cornish and Block, 1960b) 24 hours after exposure of rats to 6000 ppm of CCl_4 vapor for 6 hours serum esterase is markedly elevated. Liver esterase levels at this time have dropped well below normal values. Electrophoretic patterns of control and exposed rats are illustrated in Fig. 1. *A-1* shows the normal serum protein pattern of control rats, and *A-2* indicates the corresponding esterase pattern of the normal rat. The esterase in normal rat serum migrates near the α_2-globulin fraction of the protein and exhibits a relatively broad band of activity on the paper strip. *B-1* illustrates the serum protein distribution following CCl_4 exposure. It would appear from this pattern that there may be some alterations in the α_1- and α_2-globulin fractions. However, this altered protein pattern was not a common characteristic of all exposed animals. The albumin fraction also appears somewhat decreased in several animals. *B-2,* however, which illustrates the serum esterase distribution following CCl_4 exposure, is markedly different from the controls. Two new areas of esterase activity are present on the electrophoretic pattern obtained from the sera of exposed rats. The new areas of enzyme activity migrate near the β-globulin protein fraction. These new areas of activity stain deeply and appear comparable in intensity to the normal serum esterase band, which is still present. Forty-eight hours after exposure these new areas of esterase ac-

tivity have nearly disappeared from the serum (*C-2*). This correlates with the decrease in serum esterase activity as measured by the Gomori colorimetric technique. In addition, the normal serum esterase fraction is

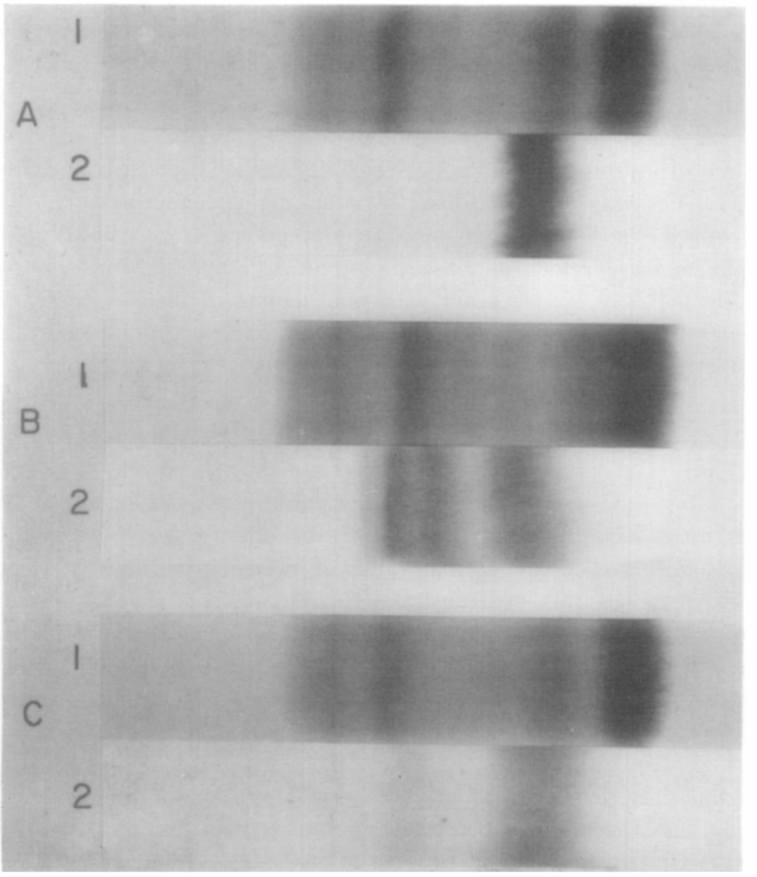

FIG. 1. Electrophoretic patterns of rat sera. *A*, control rat; *B*, 24 hours after exposure to CCl_4; *C*, 48 hours after exposure to CCl_4; *1*, protein stain; *2*, esterase stain.

noticeably decreased in the electrophoretic pattern obtained 24 and 48 hours after exposure to CCl_4 (*B-2* and *C-2*).

Control rat liver homogenates when subjected to paper electrophoresis present an esterase pattern as illustrated in Fig. *2*, *A*. There is a band of activity at the origin and two additional areas of esterase activity which

migrate toward the anode. All areas are decreased in intensity following CCl_4 exposure. None of the esterase bands in the liver electrophoretic patterns correspond in mobility with normal serum esterase (Fig. 2, *B*), which moves more rapidly under the same conditions. However, when

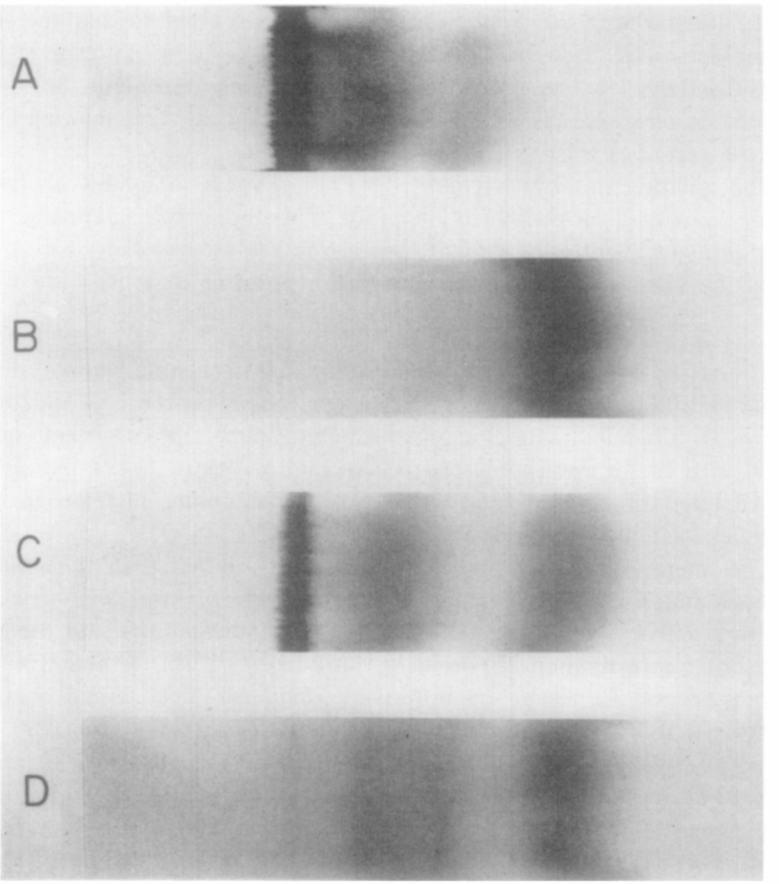

FIG. 2. Electrophoretic patterns of rat liver and rat sera stained for esterase activity. *A*, control rat liver; *B*, control rat serum; *C*, control rat liver mixed with control rat serum; *D*, rat serum 24 hours after CCl_4 exposure.

liver homogenate from control rats is mixed with control rat serum, the electrophoretic pattern obtained (Fig. 2, *C*) resembles that found in the serum of CCl_4-exposed rats (Fig. 2, *D*). The esterase activity at

the origin appears to be associated primarily with fine particulate matter in the homogenate which does not migrate on paper.

Since CCl_4 exposure is known to increase red blood cell fragility, the possibility existed that the additional bands of esterase activity found in CCl_4-exposed rats might be derived from red blood cell hemolysis. Blood from control rats was collected and homogenized so that the separated sera were deep red in color. The electrophoretic patterns of esterase activity obtained with the present staining technique on these hemolyzed sera were identical with those of control rat sera, showing only a single area of esterase activity.

The addition of physostigmine (eserine), known to inhibit cholinesterase activity, in a concentration of $10^{-4}\,M$ during the staining procedure brought about only a slight decrease in the intensity of all esterase bands in the serum and liver patterns of control and exposure rats.

DISCUSSION

The serum protein patterns obtained by this technique showed slight abnormalities in rats exposed to CCl_4 vapor. The appearance of a slightly decreased albumin fraction in some exposed animals is consistent, however, with literature reports (Berryman and Bollman, 1943) indicating a reduction of albumin and a relative and absolute increase in the globulin fraction in CCl_4-poisoned dogs.

The esterase patterns, however, are markedly different in the sera of normal and CCl_4-exposed rats. The appearance of two new areas of esterase activity in the sera of exposed rats indicates that we are not measuring an activation of normal serum esterases when we find elevated serum esterase values by quantitative techniques. The normal area of esterase activity is still present in the patterns obtained from rats 24 hours after exposure although it may be decreased in quantity. In many cases the two new areas of esterase activity which appear in the patterns of exposed rats are so broad that they merge and only a single broad new band is observed in the β-globulin area, as in Fig. 2, *D*. The question does arise as to the source of the normal serum esterase. The results obtained in these studies do not rule out the release of a tissue esterase fraction from CCl_4-exposed rats which migrates in serum to coincide with the normal serum esterase. Forty-eight hours after exposure the two new areas of esterase activity in exposed rat serum are barely detectable and, in addition, the normal area of esterase activity appears to have decreased. This is consistent with quantitative measurements

(Cornish and Block, 1960b) which indicate a drop in serum esterase activity to approximately 50% of control levels 48 hours after exposure.

The liver esterase patterns obtained in these studies show a distinct loss of esterase activity, apparently in all fractions, following exposure of rats to CCl_4 vapor. In liver homogenates the mobility of the esterase fractions do not correspond with those in the sera. However, when normal liver homogenate is added to control serum, the pattern obtained is similar to that found in CCl_4-exposed rats. While this does not show that liver is the source of increased serum enzymes it does indicate that liver esterases released into the blood stream would produce an esterase pattern consistent with that found in CCl_4-exposed animals.

It has also been demonstrated that hemolysis of red blood cells would not produce an esterase electrophoretic pattern such as that found in CCl_4-exposed rats. Since the addition of eserine to the incubation mixture during the staining procedure did not markedly alter the serum esterase pattern of CCl_4-exposed rats it would also appear that cholinesterase is not the major enzyme in any of the esterase fractions. There did appear to be some loss in the intensity of staining in all fractions in the presence of eserine, thus cholinesterase may be present in these esterase areas or this may merely represent a partial inhibition of nonspecific esterase activity.

SUMMARY

The paper electrophoretic patterns of normal rat sera contain a single band of esterase activity. The sera of rats exposed to CCl_4 exhibit two new areas of esterase activity. When normal rat serum is mixed with normal rat liver an electrophoretic pattern of esterase activity is obtained which is consistent with that found in the sera of CCl_4-exposed rats. Thus the release of liver enzymes into the blood stream appears capable of producing electrophoretic patterns containing multiple areas of esterase activity such as those found in CCl_4-exposed rats.

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